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Response of tree growth and water use efficiency to climate and nitrogen deposition in a temperate deciduous forest in the northeastern US

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RESPONSE OF TREE GROWTH AND WATER USE EFFICIENCY TO CLIMATE AND NITROGEN DEPOSITION IN A TEMPERATE DECIDUOUS FOREST IN THE NORTHEASTERN U.S.

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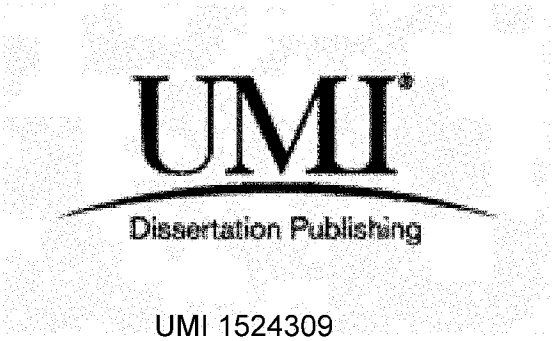

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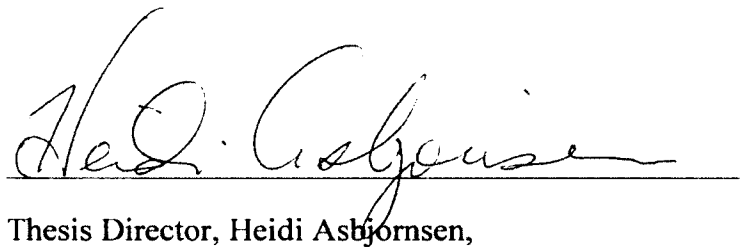

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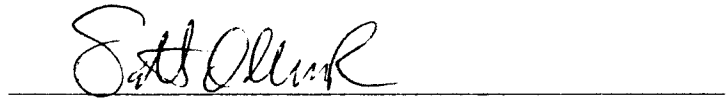

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ABSTRACT

RESPONSE OF TREE GROWTH AND WATER USE EFFICIENCY TO CLIMATE CHANGE AND NITROGEN DEPOSITION IN A TEMPERATRE DECIDUOUS FOREST IN THE NORTHEASTERN U.S.

By

Katie Ann Jennings

University of New Hampshire, September 2013

Nitrogen (N) deposition and climate change are altering tree growth and may have important consequences for water use in northeastern forests. Intrinsic water use efficiency (iWUE), the trade-off between water loss and carbon fixed during photosynthesis, directly links forest carbon and water cycles. Dendrochronology and stable isotopes ($\delta^{13}C$ and $\delta^{18}O$) were used to assess *Quercus velutina* growth and iWUE within a chronic N deposition experiment (20+ years of fertilization at low- and high-N rates). Fertilized trees exhibited sustained growth enhancement, with the greatest response observed for high-N trees. All fertilized trees improved their iWUE through increased photosynthetic rates, but was only significant in low-N trees. N fertilization also resulted in increased sensitivity of tree growth to precipitation and iWUE to relative humidity. Collectively, these results suggest that N deposition may enhance growth and iWUE in the short-term, but also induce physiological stress to drought conditions over longer time scales.

INTRODUCTION

Climate change and associated anthropogenic disturbances will unequivocally shape the future composition, diversity and productivity of our forests. Due to anthropogenic activities since industrialization, carbon dioxide (CO_2) concentrations have risen from 280 ppm to greater than 380 ppm (IPCC, 2007) and nitrogen (N) deposition has increased to unprecedented levels (Galloway et al., 2008). In New England, these two climate change drivers have already contributed to shifting forest structure regionally as many tree species, such as sugar maple (*Acer saccharum*) and red spruce (*Picea rubens*), have experienced dieback and decline (Mohan et al., 2009). The exact casual factors are difficult to pinpoint, but a combination of direct and indirect effects of climate change such as soil acidity, nutrient depletion, invasive species, and increased occurrence of freeze-thaw events and pest and disease outbreaks are believed to play a contributing role (Mohan et al., 2009 and sources therein). These trends are expected to continue to accelerate over the next century, and will likely be exacerbated by the warmer temperatures and greater frequency of extreme events such as droughts, heatwaves, and floods forecasted for New England (Douglas and Fairbank, 2011; Hayoe et al., 2008; Tang and Beckage, 2010).

The myriad of changing environmental conditions related to anthropogenic activities creates challenges in predicting the status of New England forests in the future. However, understanding tree response to various climate change drivers is a critical step in predicting and managing future change, not only because of the effect on forest health, but also because forests directly influence climate. Forest ecosystems exert feedbacks on

climate through two primary mechanisms: 1) they are the largest terrestrial biological carbon (C) sink through their role in sequestrating and storing CO_2 and 2) they regulate heat partitioning and ecosystem water balances through transpiration and canopy interception of rainfall, which determines how water moves through the soil-plant-atmosphere continuum (Bonan, 2008). Because these feedbacks can have significant consequences for regional- to global-level C and water cycles, advancing scientific understanding of these processes is crucial. Determining the degree that forests both respond to - and influence - climate change will help inform management and conservation efforts aimed at mitigating the severity of climate change and adapting to future conditions.

A useful parameter for assessing how trees respond to changes in growing conditions is 'intrinsic water use efficiency' (iWUE), or the amount of C fixed by photosynthesis per molecule of water lost via transpiration. In other words, plants that have higher iWUE can fix more C for less water than plants with lower iWUE. iWUE is influenced by multiple factors, including atmospheric CO_2 concentration, the N content of leaves, climate conditions, and individual species' physiological adaptations. An emerging and powerful technique to examine how trees have responded to past climate drivers and shed light on how they may respond under future conditions is to combine dendrochronology (the study of tree cores) with stable isotope ecology (McCarroll and Loader, 2004). The composition of stable C isotopes ($\delta^{13}C$) in wood or leaf tissue can be used as an indicator for tree WUE (Farquhar et al., 1982; McCarroll and Loader 2004). Data on stable oxygen isotope ($\delta^{18}O$) composition of plant tissue can then be combined with $\delta^{13}C$ data to determine whether any changes in tree iWUE was primarily due to

changes in rate of C uptake, transpiration, or both (Barbour, 2007). This information is especially relevant for understanding forest response to – and feedbacks on - climate change because it allows for a more detailed understanding of the relative influence of vegetation on both the C and water cycles.

New England is expected to experience intensified development of forested land over the coming decades as population densities increase in urban and suburban areas (Stein et al., 2005; Foster et al., 2010). More comprehensive estimations of future forest health and productivity in New England in response to climate and anthropogenic drivers will contribute to better understanding of potential consequences for the region's C and water cycles. This will be particularly relevant as demand for forest ecosystem services, such as water supply and flood control, should increase with continued urbanization and population growth. The overarching objective of this research was to contribute to the growing body of knowledge to better inform future projections of tree growth and health in New England forests. More specifically, this project analyzed the impacts of two regionally important global change drivers, N deposition and climate change, on the growth and iWUE of a dominant tree species (*Quercus velutina)* in a central Massachusetts forest ecosystem.

Report Organization

This report consists of two chapters. The first chapter is the primary manuscript written in format for journal publication (Global Change Biology 2014, with Rossella Guerrieri, Heidi Asbjornsen and Kathy Eggemeyer). The second chapter is a conclusion that briefly summarizes the main findings and places in a broader context of work conducted on the Chronic N Amendment Study.

CHAPTER I

Introduction

In the New England region, climate models forecast that most states can expect the average annual temperature to increase by 3.0-5.2°C and precipitation to increase by 6.8-11.4% over the next century (Tang and Beckage, 2010). It is also predicted that greater climatic fluctuations will likely lead to more frequent extended dry periods (Hayhoe et al., 2008). At the same time, human activity has increased the amount of reactive nitrogen (N) globally (Galloway et al., 2008) with Northeastern forests having experienced some of the highest deposition loads in the US (~4-12 kg N ha^{-1} yr^{-1}; Ollinger et al., 1993). Both changing climate regimes and N deposition loads may have profound effects on tree growth and has generated growing interest in predicting how forests will respond to future conditions (Allen et al., 2010; Leonardi et al. 2012; Thomas et al., 2010; van Mantgem and Stephenson, 2007). The response of trees to these global change drivers will affect forest health, distribution, and productivity, and ultimately determine the capacity of forest ecosystems to sequester C and mitigate climate change.

Among the primary responses trees exhibit to changing climate is adjustments in gas exchange at the leaf and canopy levels, which in turn, directly influences forest productivity (Hughes, 2000; Korner, 2000). Early investigations evaluating tree response to increasing atmospheric carbon dioxide (CO_2) concentrations hypothesized enhanced

growth from a CO_2 fertilization effect, but only a few studies have documented such an effect in the field (McMahon et al., 2000; Cole et al., 2010; Penuelas et al., 2011; Silva and Anand, 2013). A more pervasive global trend is that trees have exhibited either stable or declining growth rates in recent decades (Saurer et al. 2004, Gedalof and Berg, 2010; Andreu-Hayles et al., 2011). Frequently, temperature or drought-induced stress are implicated as primary underlying causes of these observed growth declines (Penuelas et al., 2008; Silva et al., 2010; Maseyk et al., 2011; Linares and Camarero, 2012), with smaller contributions from other factors such as nutrient limitation (Penuelas et al., 2011; Silva and Anand, 2013), pests and pathogens (Hicke et al., 2012), or changing fire regimes (Bond-Lamberty et al., 2007). Typically, moisture is not considered a prominent growth constraint in humid temperate region; however, this broadly held assumption has been challenged by recent studies. For example, Pederson et al. (2012) demonstrated significant drought sensitivity over long time periods (e.g., centuries) in humid forests in the Central U.S., while a meta-analysis by Choat et al. (2012) presented evidence suggesting that tree species from humid environments have similar drought sensitivity compared to dry environments. Given current climate change predictions, such extended dry periods may become more frequent and acute in coming decades.

Elevated global N deposition has typically contributed to greater forest productivity in regions where N is limited (Hogberg et al., 2006; Magnani et al., 2007; Thomas et al., 2010; Vadeboncoeur, 2010). However, the benefits of N deposition typically depend on the duration and rate of deposition (Aber, 1993; Hogberg et al., 2006). Short-term fertilization experiments or modest nutrient loads typically produce positive results with improved tree productivity (Brooks and Coulumbe, 2009; Walia et

al., 2010; Brooks and Mitchell, 2011), while long-term fertilization or elevated nutrient loads can lead to reduced tree growth and higher mortality as the system approaches N saturation (Magill et al., 1997; Magill et al., 2004; May et al., 2005).

The concept of N saturation first emerged in the 1980's when various terrestrial ecosystems demonstrated N loads in excess of biological demand (Aber et al., 1989 and sources therein). Since then, N saturation theory has developed significantly with a series of progressive steps that outlines changes in biogeochemical processes as N is chronically added to the system, forming a gradient beginning with N limited to N saturated (defined as declines in forest productivity with accelerated nitrate leaching; Aber et al., 1998; Lovett and Goodale, 2011). Subsequently, several fertilization trials were established across the U.S. and Europe (Wright and van Breeman, 1995; Wright and Rasmussen, 1998; McNulty et al., 2005; Hogberg et al., 2006; Lovett and Goodale, 2011) to experimentally test N saturation in forest ecosystems, including the Chronic N Amendment Plots at Harvard Forest in central Massachusetts (Aber et al., 1989). Past work on the Harvard Forest plots has documented dynamic changes within the stands including altered litter decay (Magill and Aber, 1998), changes in microbial community composition (Frey et al., 2004), and shifts in tree productivity and root and foliar nutrient status (Magill et al., 1997; Magill et al., 2004). The most recent comprehensive assessment of the first 15 years of fertilization indicated that N fertilization in the hardwood stand stimulated aboveground productivity, with the high-N treatment producing the greatest increases (Magill et al., 2004). However, there is evidence that these trees are highly stressed as well, with high mortality rates (Magill et al., 2004) and elevated biochemical stress markers in foliage (Minocha et al., 2000). Overall, these

studies on N saturation demonstrate widely varying tree responses to chronic N deposition across sites, and there is general consensus that N saturation processes and impacts are modulated by species composition (Magill et al. 2004), rate of deposition (Hogberg et al., 2006), and inherent site characteristics such as stand age, site history, initial nutrient status and soils capacity to cycle N through the system (Wallace et al. 2007).

One approach increasingly being used to assess tree growth and physiological responses to complex climate and anthropogenic drivers is the coupling of dendrochronological studies with stable isotope analysis. Dendrochronological studies assess woody increment growth through time, which provide one measure of a tree's ability to assimilate C under changing climate conditions. However for many forest ecosystems, particularly in humid temperate regions, radial growth is influenced by multiple factors and therefore has weak climate relationships (Loader et al., 2003; Waterhouse et al., 2004). Assessing tree physiology through analysis of stable isotopes can elucidate more direct and complex interactions between trees and their ecosystems (McCarrol and Loader, 2004). In particular, $\delta^{13}C$ can be used as a proxy to assess intrinsic water use efficiency (iWUE), the ratio of gas exchange processes between photosynthesis (A) and stomatal conductance (g_s), which can be used to derive c_i/c_a, the ratio of $[CO_2]$ between the leaf intercellular spaces (c_i) and the atmosphere (c_a). In addition, analysis of $\delta^{18}O$ can be used to trace changes in g_s, when source water and evaporative enrichment are constant among trees (Roden and Farquhar, 2012; Scheidegger et al., 2000), thereby making it possible to tease apart whether changes in A or g_s contribute more to explaining observed differences in c_i and iWUE.

Similar to productivity, increases in N supply also typically lead to an improvement in iWUE (Ripullone et al., 2004), but not always (Balster et al., 2009; Elhani et al., 2005). Many field experiments that have addressed the influence of N deposition on iWUE commonly evaluate short-term responses to fertilizer application and have mixed responses as to whether *A* (Walia et al., 2010; Brooks and Mitchell, 2011; Guerrieri et al., 2011) or g_s (Brooks and Coulombe, 2009; Guerrieri et al., 2009) was stimulated. In a uniquely long-term study, Betson et al. (2007) analyzed foliar $\delta^{13}C$ from Scots pine (*Pinus sylvestris*) that received elevated N fertilization for 32 years. Fertilized trees exhibited an initial increase in $\delta^{13}C$ that was maintained for 32 years, but no additional changes in iWUE were observed. However, species differ in their response to increases in N supply. For instance, conifers are known to invest less N to producing photosynthetic enzymes (i.e. Rubisco) than deciduous species (Ripullone et al., 2004; Wullschleger, 1993). However, similar long-term N fertilization experiments on deciduous species are lacking, representing a critical research need.

The main purpose of this study was to evaluate the growth and iWUE of a common temperate forest deciduous species, *Quercus velutina,* in response to long-term elevated N fertilization and climate variability. Trees were sampled from a Chronic Nitrogen Amendment study at Harvard Forest that received annual N fertilization for more than two decades. This study used a coupled dendrochronology-stable isotope approach to assess the long-term patterns in *Q. velutina* growth and iWUE in response to N fertilization and climate parameters. Our specific objectives were to assess how N fertilization influences tree growth and iWUE, the primary mechanism by which iWUE is influenced by N fertilization (i.e., by altering either *A* or g_s), and the effect of interactions

between N fertilization and climate on tree growth and iWUE. Our specific hypotheses were as follows:

(1) Following implementation of the nutrient amendment treatments, trees initially experienced increased growth and an improvement in iWUE relative to the control plot due to enhanced foliar N concentration and, hence, stimulation of photosynthetic capacity (*A*).

(2) Over time, stimulation in growth and iWUE declined as excessive N load begin to negatively impact tree health due to effects of N saturation.

(3) iWUE and growth increment are more strongly influenced by local site factors and N deposition than by fluctuations in climate; however, exceptions may occur during extreme climatic events (such as severe drought).

Materials and Methods

Site Description

The Chronic Nitrogen Amendment Study at Harvard Forest in central Massachusetts (42°30' N, 72°10' W) was established in 1988 as part of a Long-Term Ecological Research Program to examine the impact of N saturation on the health and productivity of temperate forest ecosystems (Aber et al., 1993). Two stands were selected for the study, a 70 year old red pine *(Pinus resinosa)* plantation and an approximately 60+ year-old (Magill et al., 2000) mixed hardwood stand dominated by black and red oak (*Quercus velutina* Lam. and *Q. rubra* L.) with significant amounts of black birch (*Betula lenta* L.), red maple (*Acer rubrum* L.) and American beech (*Fagus grandifolia* Ehrh.). The dominant soils for both stands are stony to sandy loam formed from glacial till. The

sites have an average annual temperature of 13°C and receive approximately 1120 mm of precipitation (Aber et al., 1993; Magill et al., 1997). The ambient wet and dry N deposition rate for this region is about 8 N $ha^{-1}yr^{-1}$ (Ollinger et al., 1993).

The stands are divided into four 30 x 30 m plots: low-N, high-N, low-N plus sulfur (S) and a control plot. The plots were fertilized with a dilution of ammonium nitrate or ammonium nitrate sodium sulfate, mixed with water and applied six times a year from 1988 to 2004. Starting in 2005, ammonium nitrate plus potassium fertilizer has been used. The low N plot receives 50 kg N $ha^{-1}yr^{-1}$ and the high N plots receives 150 kg N $ha^{-1}yr^{-1}$, (Magill et al., 1997), hereafter 'low-N', 'high-N' and 'control' plot, respectively.

Field Sampling and Dendroclimatic Assessment

Ten healthy dominant *Q. velutina* trees were sampled each from the control, low- and high-N plots in the hardwood stand during October of 2011. Three cores per tree were taken at breast height perpendicular to slope with a 5.15 mm increment borer. Cores were mounted, sanded, skeleton plotted, and cross-dated following standard dendrochronological procedures (Fritts and Swetman, 1989). The rings were measured to 0.001 mm precision with a sliding scale micrometer (Velmex Measuring System). The ring series were than validated in COFECHA, a statistical cross-dating quality control program (Holmes, 1983). Basal area increment (BAI) series were calculated from the total ring widths using the equation:

$$BAI = \pi(R_n^2 - R_{n-1}^2) \quad (1)$$

where R is the radius and n is the year of tree ring formation.

To develop the chronology for each plot, ring width series were standardized in ARSTSAN using a 30-year moving cubic smoothing spline that preserved 50% of the variance to remove trends from the series that were not climatic in origin, such as trends related to tree age (Cook, 1985). ARSTAN produces three ring indices; the ARSTAN (ARS) index attempts to preserve the strongest climate signal by reincorporating pooled autoregression into the residual chronology (Cook and Holmes, 1986).

Stable Isotope Analysis

Five trees from each plot were selected based on highest confidence of dating accuracy for subsequent isotope analysis. Annual rings were divided into earlywood and latewood sections, and the latewood for each year was pooled by tree (one sample per tree per year). To minimize samples, while still encompassing the entire fertilization period at annual resolution, groups of years were selected for analysis: 1984-1993 (ten years surrounding the onset of fertilization at 1988), 1998-2002, and 2008-2011, for a total of 285 samples (3 treatments x 5 trees x 19 years). Wood samples were ground and then extracted for holocellulose ($\delta^{13}C$) or α-cellulose ($\delta^{18}O$) (Leavitt and Danzer, 1993; Sternberg, 1989). For $\delta^{13}C$ analysis, 1-3 mg of holocellulose of each sample was weighed into tin capsules and combusted on an elemental analyzer (Costech 4010 Elemental Analyzer) coupled to an isotope ratio mass spectrometer (Delta Plus XP Mass Spectrometers). For $\delta^{18}O$, 0.15-0.25 mg of α-cellulose of each sample was weighed into silver capsules and analyzed on an elementar PyroCube (Elementar Analysensysteme

GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Relative stable isotope abundances are expressed as ratios of $^{13}C/^{12}C$ and $^{18}O/^{16}O$ using δ-notation (in per mil; ‰) in reference to an established standard (VPDB for δ ^{13}C and VSMOW for δ ^{18}O).

$$\delta sample = \left[\frac{\delta sample}{\delta standard} - 1\right] 1000 \quad (2)$$

Stable Isotope Theory

Carbon Isotope and iWUE - The $\delta^{13}C$ composition of the wood is generally depleted relative to the atmospheric ratio due to the biophysical and biochemical processes that discriminate against ^{13}C during C fixation. Wood $\delta^{13}C$ represents the $[CO_2]$ ratio between the leaf intercellular spaces (c_i) and the atmosphere (c_a) after it has undergone several known fractionations:

$$\delta^{13}C = a + (b - a)\left(\frac{c_i}{c_a}\right) \quad (3)$$

where a is the fractionation due to diffusion (4.4‰) and b is the fractionation due to carboxylation by Rubisco (~27‰) (Farquhar et al., 1982). iWUE is the direct trade-off between net photosynthesis (A) and water loss due to stomata conductance (g_s):

$$iWUE = \frac{A}{g_s} = \frac{(c_a - c_i)}{1.6} \quad (4)$$

where 1.6 is the ratio of diffusivities of water and CO_2 in the air. Thus, the ^{13}C isotopic signature from tree rings can trace the variations in iWUE through time with respect to rising atmospheric [CO_2] and other climatic or site factors.

Oxygen Isotope - Whereas variation in $\delta^{13}C$ reflects a change in A, g_s or both, the $\delta^{18}O$ composition of wood is related to changes in leaf water $\delta^{18}O$ in response to transpiration. When source water and atmospheric water vapor are constant among sites, the $\delta^{18}O$ of wood tissue can represent variations in g_s. During transpiration within the leaf, the lighter $H_2{}^{16}O$ molecules evaporate faster than the heavier $H_2{}^{18}O$ molecules, creating an enriched ^{18}O water within the leaf. The isotopic composition at the site of evaporation ($\delta^{18}O_e$) is expressed as the difference between the intercellular (e_i) and atmospheric (e_a) vapor pressure, as described by Craig and Gordon (1965):

$$\delta^{18}O_e = \delta^{18}O_s + \varepsilon^* + \varepsilon_k + (\delta^{18}O_v - \delta^{18}O_s + \varepsilon_k)\frac{e_a}{e_i} \quad (5)$$

where $\delta^{18}O_v$ and $\delta^{18}O_s$ represent the isotope composition of water vapor and source water, respectively, ε^* is the equilibrium fractionation factor and ε_k is the kinetic fractionation as water diffuses through the stomata and boundary layer. Bulk leaf water is a mixture of evaporated and source water, while the isotopic signature of bulk leaf water ($\delta^{18}O_l$) can be calculated as (Barbour, 2007):

$$\delta^{18}O_l = \frac{\delta^{18}O_e(1-e^{-\wp})}{\wp} \quad (6)$$

where $\wp$ represents the Péclet effect, a ratio of convection to diffusion. The composition of cellulose ($\delta^{18}O_{cel}$) can be calculated as (Barbour and Farquhar, 2000):

$$\delta^{18}O_{cel} = \delta^{18}O_l(1 - p_{ex}p_x) + \varepsilon_o \quad (7)$$

where p_{ex} is the proportion of oxygen atoms that exchange with source water during cellulose formation, p_x is the proportion of unenriched (source) water at the site of cellulose formation, and ε_o is the fractionation factor due to exchange between carbonyl oxygen and water.

Climate Data

Data for mean monthly precipitation, temperature and the Palmer Drought Severity Index (PDSI) for the State of Massachusetts were collected from the National Oceanic and Atmospheric Administration (NOAA) databases. Additional climate data from the Harvard Forest Shaler (1964-2002) and Fisher (2001-2011) meteorological stations were also collected for precipitation, temperature, maximum temperature and minimum temperature. Atmospheric CO_2 and $\delta^{13}C$ concentrations prior to 2004 were obtained from McCarroll and Loader (2004) and for the period 2004 to 2011 from Mauna Loa.

Data Analysis

Ring widths- Bootstrapped correlation and response function coefficients were calculated between monthly climate data (16 month growing seasons from previous June

through current September) and the ARS ring width index using the software package DENDROCLIM2002 (Biondi and Waikul, 2004). This software calculates the 95% quantile limits based on 1000 bootstrap re-samples of the data to test for the statistical significance of the correlation and response coefficients. Only monthly climate variables with both significant correlations and response coefficients are reported in the results. Treatment differences in mean BAI were tested pre- and post- onset of fertilization using ANOVA.

Isotopes- Two main issues confounded the analyses of these data 1) the treatment plots were unreplicated and 2) there was evidence of pretreatment differences among the five trees selected for isotope analysis. To account for this, response ratios (RR), normalized for pre-treatment differences were calculated. RR is defined as the ratio of a response from a treatment group relative to the control to quantify the proportional change from an experimental manipulation (Hedges et al., 1999). RR were calculated as follows:

$$ln(RR) = ln\frac{X_E}{X_C} \quad (8)$$

where X_E represents the BAI or iWUE in the high or low fertilized plot after correction for pretreatment differences and X_C is the response in the control plot. Student's *t*-test was used to test for significant responses. Response variations for isotopes, iWUE , BAI and foliar N content were evaluated by calculating percent change compared to the pretreatment values within treatments, and significant changes through time were evaluated using ANOVA. Simple linear regression was used to evaluate the relationship between iWUE vs BAI and $\delta^{18}O$. Additionally, $\delta^{13}C$, $\delta^{18}O$ and iWUE responses to

climate were estimated using multiple linear regression for 1) summer (May-September) mean monthly precipitation and temperature and 2) summer climate with cumulative N fertilization. The focus on *summer* climate was selected as regressions with other months (e.g. from winter or spring) were irregular and trends were difficult to detect. Unless otherwise specified, all statistical analyses were conducted in JMP statistical software (Version 10, SAS Institute Inc., Cary NC, 1989-2012).

Results

Growth Response to Fertilization and Climate

The ring width chronologies developed for the *Q. velutina* trees from the control, low- and high-N plots were similar in age and summary statistics (Table 1). The mean chronology ranged from 50.0-59.6 years reflecting a similar period of establishment in the 1940's after the 1938 hurricane (Magill et al., 2004). The interseries correlation (Rbar) and expressed population signal (EPS) value shows there is a common growth signal among the trees indicating accurate dating and high chronology reliability. The relatively low mean sensitivity for these chronologies demonstrate low inter-annual variation in growth, indicating that these *Q. velutina* trees are fairly complacent and are overall not very sensitive to climate.

Table 1. Summary statistics for *Q. velutina* tree ring standardization. Mean chronology is the average number of years for three cores per tree. Rbar is the average correlation among trees for the common overlap period among series (Cook and Kairiukstis, 1990). MS (mean sensitivity) is the average difference between successive ring width values, ranging from 0-2 with 0 representing no difference between successive ring widths and 2 representing every second ring missing (Fritts, 1976; Tardif et al., 2006). EPS (expressed population signal) is an indicator of chronology reliability by comparing the chronology with the theoretical chronology based on an infinite number of trees (Cook and Kairiukstis, 1990; Tardif et al., 2006). EPS ranges from 0.0-1.0 from no agreement to perfect agreement with the population chronology. Autocorrelation is the correlation among the ring width series before transformation.

Treatment	**Mean chronology (years)**	**Rbar**	**MS**	**EPS**	**Autocorrelation**
Control	59.6	0.585	0.202	0.934	0.771
Low (50 kg/ha/yr)	50.0	0.667	0.199	0.952	0.731
High (150 kg/ha/yr)	54.8	0.697	0.195	0.958	0.740

Before the onset of fertilization, the *Q. velutina* trees across all plots grew similarly, following comparable patterns in basal area increment through time including a large reduction in biomass production in the early 1980's (Fig. 1). After fertilization began in 1988, trees from the low- and high-N plots had significantly higher growth rates over the control ($p > 0.0001$), while trees in the high-N plot grew at a faster rate than trees in the low-N plot ($p < 0.01$). Specifically, over the entire 23 years of fertilization, the average cumulative BAI was 118, 192 and 222 $cm^2\ tree^{-1}$ in the control, low-N and high-N plot, respectively, which reflects cumulative increases of 62% (low-N) and 88% (high-N) relative to the control plot.

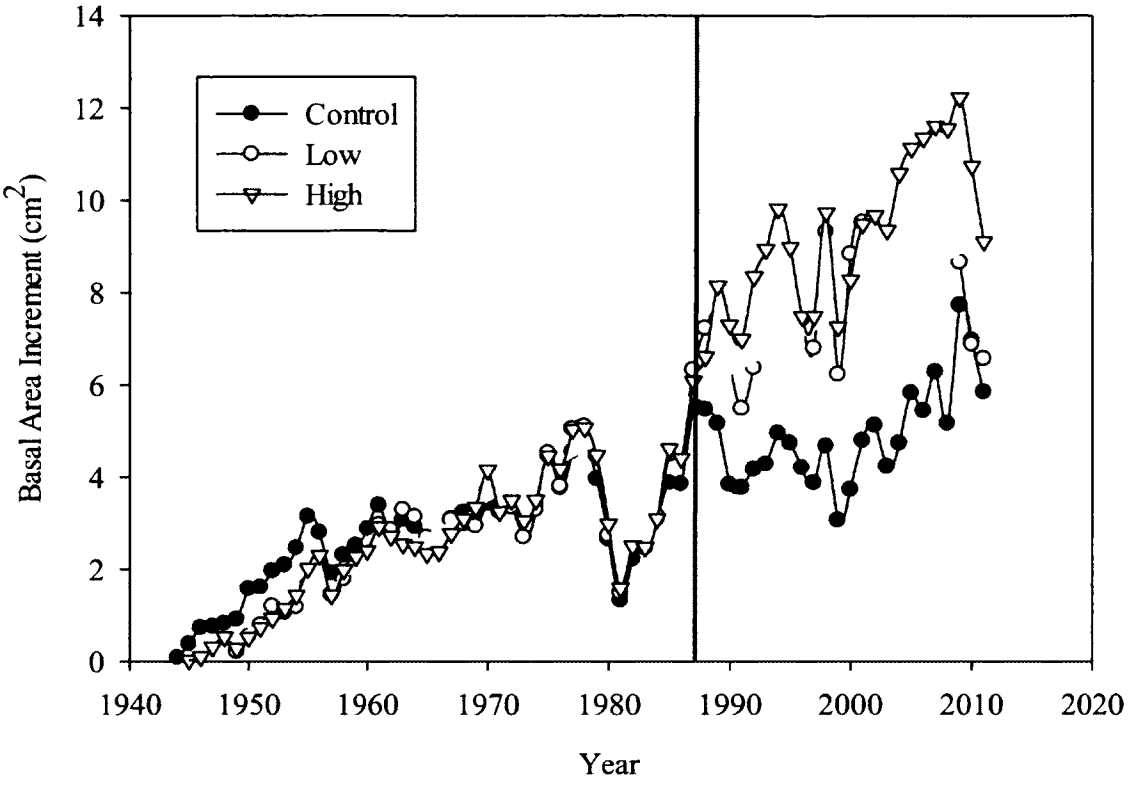

Figure 1. Average basal area increment for ten healthy *Q. velutina* trees by treatment. Black vertical line represents 1988, the year annual fertilization began.

Overall, the *Q. velutina* trees examined in this study exhibited a relatively weak relationship between woody increment growth and climate (Table 2). The significant response function relationships were primarily associated with the previous growing season's summer months, especially July (pJuly, Table 1). Additionally, increment growth was consistently sensitive to previous July maximum temperatures across all treatments, while trees in the two fertilized plots also exhibited a weak relationship with summer precipitation (previous July for the low-N plot, and previous and current August for the high-N plot).

Table 2. Significant response coefficients ($p < 0.05$) for the relationship between *Q. velutina* growth and monthly climate parameters by treatment and climate dataset. pJuly and pAugust represent July and August from the *previous* growing season.

Climate Data Set	Climate Parameter	Control	Low	High
Harvard Forest	Prec		0.247 (pJuly)	0.262 (August)
	Max T	-0.249 (pJuly)	-0.258 (pJuly)	-0.248 (pJuly)
MA NOAA	Prec			0.282 (pAugust)

Physiological Response to Fertilization

Over the study period, *Q. velutina* trees growing in the N fertilization treatment plots exhibited higher iWUE, BAI, and $\delta^{18}O$ and less negative $\delta^{13}C$ compared to the control, although the difference varied by time period (T1-T3, Fig. 2). Prior to fertilization, differences existed among the three plots (T0, Fig. 2), most notably, cellulose of trees growing in the low and high-N plots was significantly more enriched in $\delta^{18}O$ than trees growing in the control plot, $p < 0.001$). Given the pretreatment differences, significant responses among treatments were evaluated in the following ways: 1) temporal changes, analyzed within treatment (relative to pretreatment values; Table 3) and 2) response ratios (RRs), normalized for pretreatment differences, comparing the fertilized plots relative to the control (Fig. 3). Temporally, all three plots showed similar trends in isotopic composition. When comparing T1 and T2 relative to pretreatment (T0) conditions, $\delta^{13}C$ increased across all plots, while $\delta^{18}O$ either increased (control plot) or remained constant (fertilized plots). However, a significant ($p < 0.001$) depletion in $\delta^{18}O$ relative to T2 was observed during T3, along with a concurrent depletion in $\delta^{13}C$, which combined are indicative of a strong climatic driver, which may have increased stomatal conductance, thereby explaining the increase in c_i (Fig. 2, Table

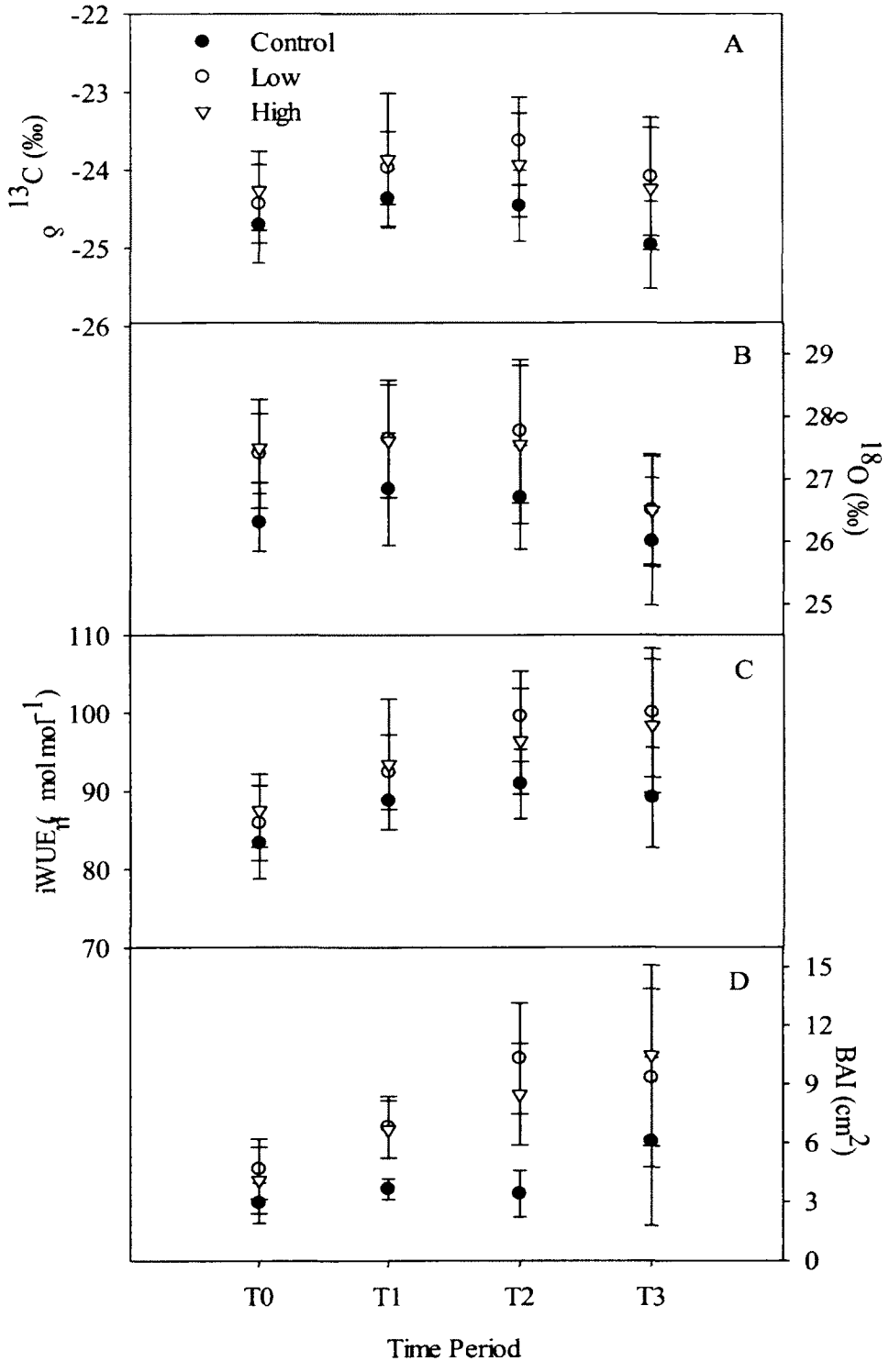

Figure 2. Trends in *Q. velutina* (A) iWUE, (B) BAI, (C) $\delta^{13}C$ and (D) $\delta^{18}O$ during the study period. Symbols represent mean response (± SD) of five trees per treatment for each time period: T0 (pre-fertilization, 1984-1987), T1 (1988-1993), T2 (1998-2002) and T3 (2008-2011).

3). In the fertilized plots, iWUE remained high during T3 despite an increase in g_s, indicating that A was stimulated to offset the change in stomatal conductance.

Table 3. Percent (%) change for $\delta^{13}C$, $\delta^{18}O$, iWUE and BAI for each treatment plot relative to the pretreatment values by time period following onset of fertilization treatment. Bolded values are significantly different from the pre-treatment values (T0), and the letters represent levels of significance (from T0) as determined by ANOVA. T1 (1988-1993), T2 (1998-2002) and T3 (2008-2011).

Percent (%) Change		Control	Low	High
$\delta^{13}C$	T_1	**-1.35 b**	**-1.88 b**	-1.64 a
	T_2	-0.97 a	**-3.29 c**	-1.34 a
	T_3	1.07 a	-1.41 a	-0.09 a
$\delta^{18}O$	T_1	2.02 a	0.86 a	0.42 a
	T_2	**1.50 b**	1.29 a	0.20 a
	T_3	-1.17 a	**-3.31 b**	**-3.63 b**
iWUE	T_1	**6.49 b**	**7.57 b**	**6.77 b**
	T_2	**9.09 b**	**15.89 c**	**10.16 b, c**
	T_3	**7.01 b**	**16.44 c**	**12.39 c**
BAI	T_1	24.31 a	**46.10 b**	**63.40 b**
	T_2	16.07 a	**120.99 c**	**107.03 c**
	T_3	**106.91 b**	**99.74 c**	**155.50 d**

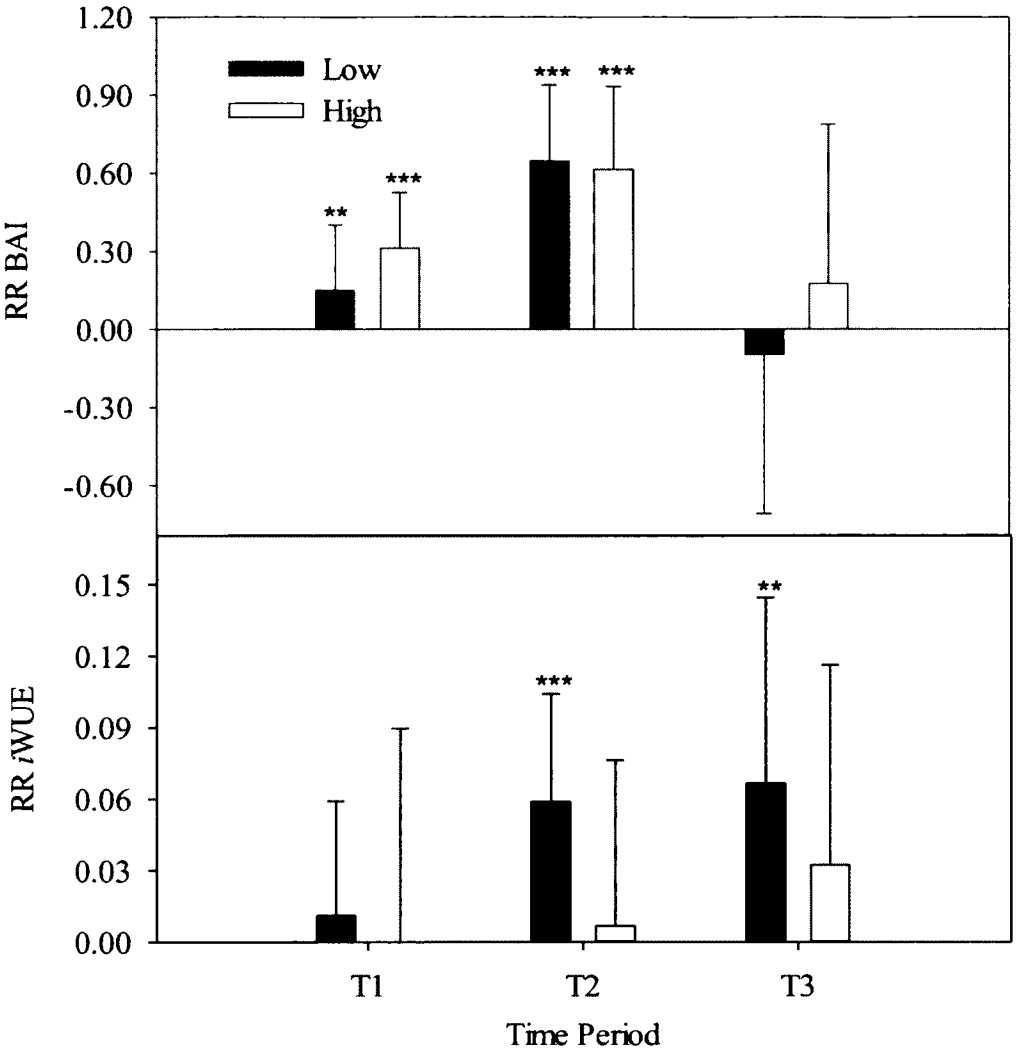

Figure 3. Response ratios normalized by pretreatment values (± SD) for BAI and iWUE over the three time periods after onset of fertilization treatment. T1 (1988-1993), T2 (1998-2002) and T3 (2008-2011). Stars represent level of significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Based on the response ratios, BAI was initially greater for trees growing in the low- and high-N plots compared to the control during T1 and T2; however, this difference disappeared during T3 due to a large increase in the control trees' BAI (a doubling of BAI relative to pre-treatment BAI, Table 3). Significant increases in iWUE in response to the N fertilization treatment were only detected in the low-N plot during

T2 and T3 (Fig. 3B); notably, iWUE for trees growing in the control plot also increased over the study period (Table 3). Significant trends were generally difficult to detect since the fertilized trees showed considerable variability between individual trees, as can be seen from the large standard deviations (Fig. 2 and Fig. 3).

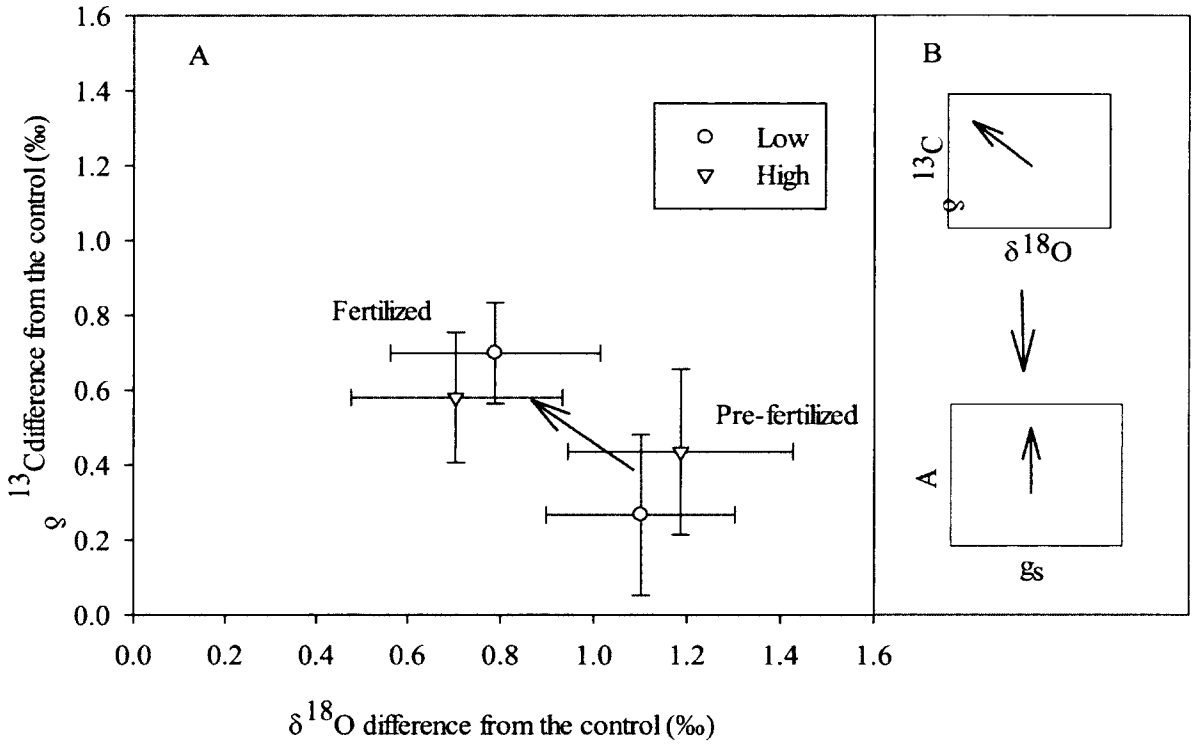

Figure 4. (A) Summary of $\delta^{18}O$ vs $\delta^{13}C$ trends for the low and high treatment plot represented as difference from the control (treatment value-control value), grouped into pre-fertilized (1984-1987) and fertilized (1988 - 2011) time periods. Bars represent 95% confidence intervals. (B) represents the directionality of the $\delta^{18}O$ vs $\delta^{13}C$ relationship and most probable change in A_{max} vs g_s according to Schiedegger et al., 2000.

To better understand the isotopic response of the trees in the N fertilization treatments, the difference between the treatment and control $\delta^{13}C$ and $\delta^{18}O$ values were analyzed to remove the influence of climate and site conditions (Fig. 4). The $\delta^{18}O$ vs $\delta^{13}C$ relationship is broadly grouped into pre-fertilized and fertilized time periods for the low-

and high-N plots to summarize the overall treatment effect. Both of the treatment plots follow the same general pattern of decreasing $\delta^{18}O$ with accompanying increases in $\delta^{13}C$ over the fertilization period, although the slope of the relationship is different between fertilization rates. Trees in the low-N plot had a smaller change in $\delta^{18}O$ and greater change in $\delta^{13}C$ (i.e. steeper slope) compared to the high-N trees. By applying the conceptual model developed by Scheidegger et al. (2000), we can predict the most probable change in A_{max} and g_s that produced changes in iWUE by looking at the directional relationship of $\delta^{18}O$ vs $\delta^{13}C$. This conceptual model infers changes in c_i from $\delta^{13}C$ and changes in relative humidity (RH) from $\delta^{18}O$ which is then use to determine the directional shifts in g_s. Information about the relative changes in RH and g_s is then used to infer the probably response of A_{max}. This model assumes that source water and RH is constant among study trees. In this case, the model predicts a stimulation of A_{max} to be the dominant driver increasing iWUE (Fig. 4b). This is supported by other results suggesting increased C assimilation and productive capacity for the fertilized trees including 1) significant increases in BAI (Fig. 1, 3), 2) significantly enhanced foliar N content (Table 4), and 3) positive relationship between iWUE and BAI (Table 5).

Table 4. Percent (%) change foliar N content for each treatment plot relative to 1988 values by time period following onset of fertilization treatment. Bolded values are significantly different from the 1988 values, and the letters represent levels of significance as determined by ANOVA. T1 (1988-1993), T2 (1998-2002) and T3 (2008-2011). Data from Frey and Ollinger, Harvard Forest Data Archives: HF008.

	Control	**Low**	**High**
T1	11.1 a	11.4 a	**29.3 b**
T2	18.5 a	**30.6 b**	**52.9 c**
T3	17.7 a	**26.0 b**	**45.2 b, c**

Table 5. Linear regression between iWUE vs $\delta^{18}O$ and BAI. r^2, slope coefficient (b) and significance from F test. Stars represent level of significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

	$d^{18}O$		**BAI**	
	r^2	b	r^2	b
Control	**0.118*** **	**2.10**	**0.146*** **	**0.86**
Low	0.014	0.88	**0.210*** **	**1.07**
High	0.019	-1.09	**0.078** **	**0.65**

Physiological response to climate-N fertilization interactions

Table 6. Results from multiple linear regression of summer climate and summer climate with annual cumulative fertilization (Ncum) for each treatment predicting $\delta^{13}C$, $\delta^{18}O$ and iWUE. Modeled adjusted r^2 and F ratio from the F-test are reported. Bold models are significant, with stars designating level of significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$); ns is not significant.

Model		**Control**	**Low**		**High**	
		Climate	Climate	Climate +Ncum	Climate	Climate +Ncum
$\delta^{13}C$	**r^2**	**0.344**	**0.138**	**0.192**	-0.027	-0.037
	F	**<4.299*** **	**2.119** **	**2.519***	ns	ns
$\delta^{18}O$	**r^2**	**0.206**	0.130	**0.189**	**0.415**	**0.420**
	F	**2.847** **	ns	**2.294***	**5.969*** **	**5.627*** **
iWUE	**r^2**	0.076	0.093	**0.362**	-0.105	-0.007
	F	ns	ns	**4.611*** **	ns	ns

To better understand the influence of climate and N fertilization on $\delta^{13}C$, $\delta^{18}O$ and iWUE, multiple linear regression using mean monthly summer precipitation and temperature variables both with and without an annual cumulative fertilization term was carried out (Table 3). A decreasing sensitivity of $\delta^{13}C$ to climate as fertilization rate increases was observed for both the low- and high-N plots. Trees in the control plot showed the strongest relationship to climate parameters ($p < 0.001$), while the relationship was weaker in the low-N plot trees ($p < 0.05$) and non-significant in the high-N trees (Table 3, Fig. 5). The pattern for $\delta^{18}O$ was different; with the influence of climate

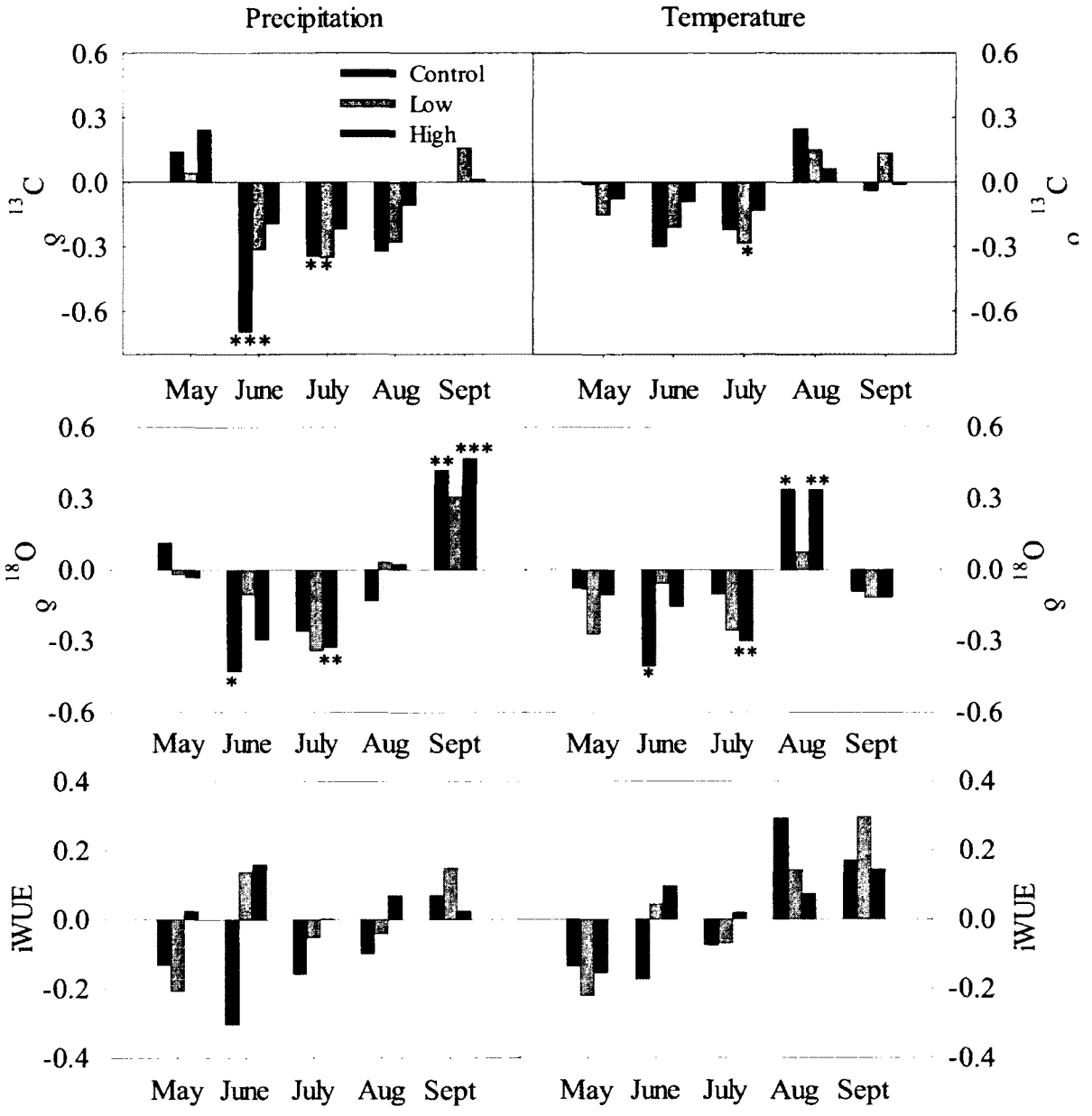

Figure 5. Multiple linear regression by treatment with summer precipitation and temperature predicting $\delta^{13}C$, $\delta^{18}O$ and iWUE. Bars represent standardized slope coefficients of each climate parameter. Climate predictors are starred when 1) the summer climate regression model is significant and 2) the specific climate variable predicts significant variation in $\delta^{13}C$, $\delta^{18}O$ or iWUE (also refer to Table 4). (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

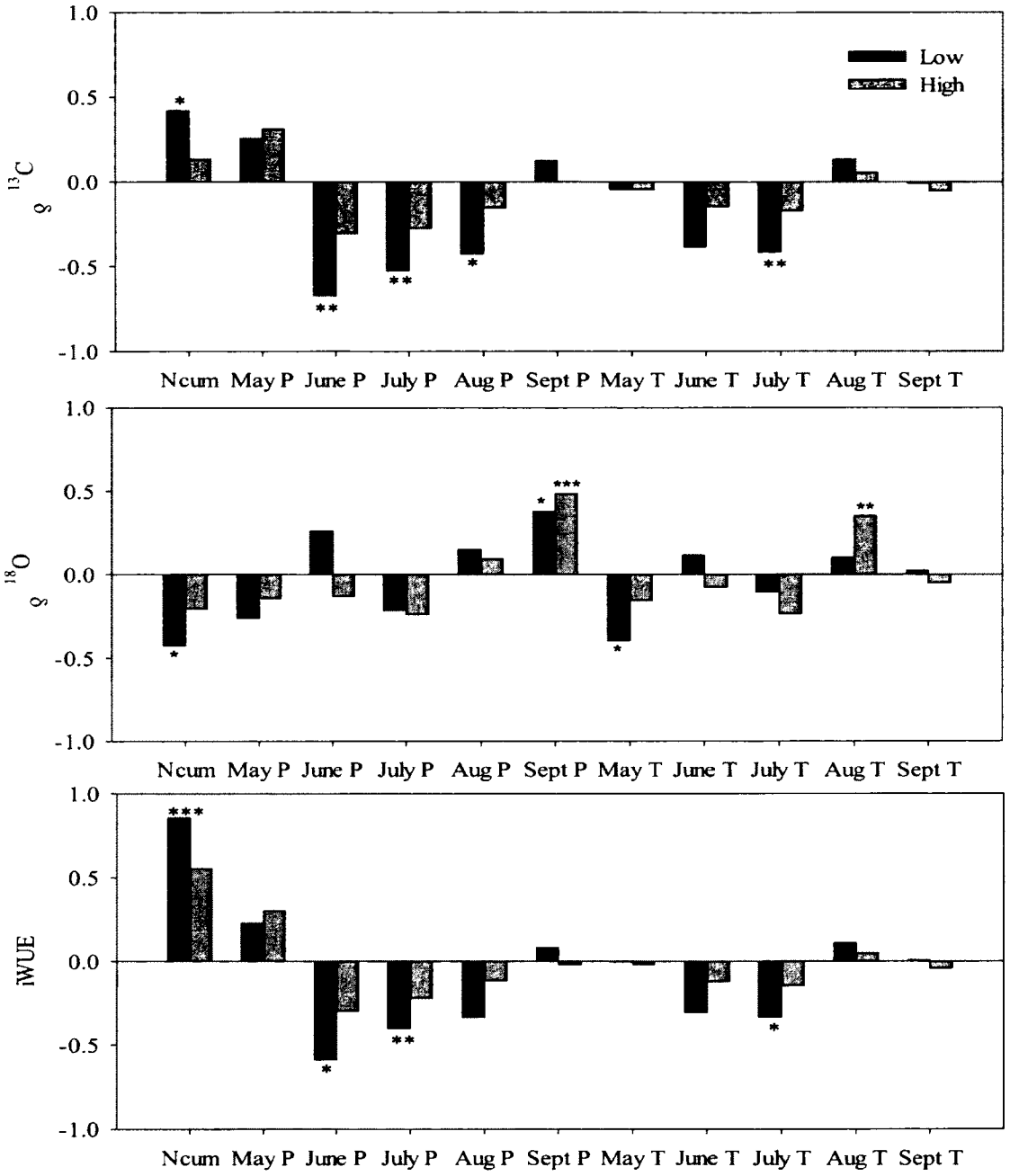

Figure 6. Multiple linear regression by treatment with summer precipitation and temperature with cumulative N fertilization (Ncum) predicting $\delta^{13}C$, $\delta^{18}O$ and iWUE. Bars represent standardized slope coefficients of each climate parameter. Climate predictors are starred when 1) the climate and Ncum regression model is significant and 2) the specific variable predicts significant variation in $\delta^{13}C$, $\delta^{18}O$ or iWUE (also refer to Table 4). (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

on ^{18}O discrimination being moderate in the control plot ($p < 0.01$), very strong in the high-N plot ($p < 0.0001$), and absent in the low-N plot. No significant relationship was found between iWUE and climate for any plot.

With the cumulative fertilization term (Ncum) added into the multiple regression model the relationship to climate improves in the low-N trees, but not for the high-N trees (Fig. 6). In the low-N trees, the $\delta^{13}C$ model with Ncum explained a larger proportion of the variance within the data (adjusted r^2 increased from 0.138 to 0.192) and two additional climate variables were detected as significant regressors (June and August precipitation). Similarly, with the addition of the Ncum term, both the $\delta^{18}O$ and iWUE models for the low-N trees were significant and followed similar patterns as those observed for the control and high-N trees, with the $\delta^{18}O$ regression showing significant relationships with late fall precipitation (positive) and early summer temperature (negative). The pattern for the iWUE model was similar to the $\delta^{13}C$ model, with negative associations observed between iWUE and both mid-summer precipitation (June-July) and temperature (July). In the high-N plot, however, inclusion of the Ncum term had very little effect on the relationship between the stable isotopes and iWUE with climate. The only significant model, $\delta^{18}O$, explained approximately the same about of variability (adjusted r^2 increasing from 0.415 to 0.420) but reduced the number of significant climate variables (Fig. 6).

Precise associations between monthly climatic variables are difficult to summarize across all treatments, but generally the *Q. velutina* trees showed negative associations with early- to mid-summer precipitation and temperature (May-July) for

$\delta^{13}C$ and $\delta^{18}O$ and positive associations with late summer climate (August-September precipitation and temperature) for $\delta^{18}O$ (Fig. 5 and 6). This translates to lower iWUE with a warm and wet early- and mid-growing season, but the relationship reverses at the end of the summer, when observed higher iWUE and $\delta^{18}O$ may be related to higher temperature and greater precipitation leading to a reduction in stomatal conductance.

Discussion

The present study offers a unique long-term assessment of tree growth and physiological responses of a central New England dominant temperate forest tree species, *Q. velutina,* to chronic N deposition and climate interactions. This builds upon past research evaluating tree growth conducted on the Chronic N Amendment plots at Harvard Forest, but also assesses other aspects that previous studies have been unable to capture, including pre-treatment growth patterns. The most recently published work from this site evaluating tree growth response to 15 years of N fertilization, observed a sustained enhancement in net primary production relative to the control in the hardwood stand, while the magnitude of this growth increase was much greater than reported by other studies of similar scope (e.g. Wallace et al., 2007 for oak trees from ridge-top forest in New York or Magill et al., 2004 for pine trees at Harvard Forest). In the present study, the fertilized trees continue to have an increase in productivity over the control through 2011 (23 years of enhancement), although the effect may be tapering off in the last 3-4 years.

Dendroclimatic analysis: Growth response of *Q. velutina* to N fertilization and climate

In contrast to most other fertilization studies (including past work on the Harvard Forest N amendment plots), the present study focused on healthy dominant trees to ensure high core quality and dating accuracy, and therefore avoided dead or dying trees. Because this approach results in an overall optimal estimation of growth response to the treatments, we emphasize the need for caution when comparing our findings with other similar studies. Nevertheless, the *Q. velutina* trees in the low- and high-N plots had significantly higher rates of woody increment growth compared to trees growing in the control plot. This effect is not surprising since past work on these plots has shown that fertilization stimulates tree productivity, including increased stem wood growth (Magill et al., 2004). This is comparable to other studies that have assessed the response of *Quercus* and other deciduous tree species to N fertilization in the Northeastern U.S. (Elvir et al., 2003; Wallace et al., 2007; Vadeboncoeur, 2010). However, the relationship is complicated by concomitant increased mortality rates, especially in the high-N plot (Magill et al., 2004; Wallace et al., 2007), variable responses among species (Magill et al., 2004; Elvir et al., 2003), and different responses depending on fertilization rate and duration (Magill et al., 2004; Hogberg et al., 2006).

Prior to fertilization, *Q. velutina* tree growth was similar across the plots, suggesting they were responding to common local disturbances or climate conditions. This growth pattern included a substantial decline across all trees in the early 1980's that can be traced to a gypsy moth epidemic that peaked in 1981(Liebhold et al., 2000). After

fertilization treatments began, Magill et al. (2004) detected decreasing growth and increasing mortality in the high-N trees between 1996-2002 indicating that benefits from high fertilization were waning and suggesting potential negative effects on forest health. In the present study, the growth increment begins to diminish between 2008-2011 in both the low- and high-N plots (Fig. 1), suggesting that even the healthiest trees may be experiencing growth declines and potentially reaching a saturation threshold.

The *Q. velutina* trees examined in this study exhibited a relatively weak relationship between growth and climate. Our analysis of significant climate response functions showed that the strongest relationship was a negative response to maximum temperature and a positive response to precipitation, primarily during the previous growing season's late summer months (Table 2). Other studies on *Quercus* spp. have documented a similar sensitivity to previous summer and fall temperature and precipitation conditions, including in South Carolina (Jacobi and Tainter, 1988; Crawford, 2012), Quebec (Tardif et al., 2006), and France (Michelot et al., 2012). Many authors have interpreted this growth-climate relationship as reflecting a high degree of vulnerability of *Quercus* trees to drought late in the previous growing season (Tardif et al., 2006; Michelot et al., 2012). *Quercus* species are susceptible to high rates of embolism during the winter (Cochard and Tyree, 1990), so large C reserves for the next year's growth are crucial for producing new xylem tissue to establish hydraulic conductivity before leaf expansion (Michelot et al., 2012). Previous late summer and fall growing conditions have been shown to influence the amount of C reserves produced and stored by *Quercus* trees (Fritts, 1976; Crawford, 2012).

The lack of other significant growth-climate correlations in our study is contrary to findings from most other related studies, which typically report multiple correlations between *Quercus* growth response and climate drivers throughout the year, including current growing season conditions (Jacobi and Tainter, 1988; Tardif et al., 2006; Crawford, 2012; Michelot et al,. 2012; Zang et al., 2012). The limited correlations to climate in the present study is likely due to the multiple confounding factors that influenced tree growth at Harvard Forest, particularly influences from local disturbances and stand dynamics. The underlying assumption of dendroclimatology is that annual ring growth in the study trees are primarily influenced by top-down climate drivers (Cook, 1992; Crawford, 2012). Hence, the ideal study site would possess low competition and strong climate pressure such as open canopied forests (Crawford, 2012) or sites located at extreme elevations or at the edge of a species' range (Phipps, 1982; Tardif et al., 2006; Speer et al., 2009). In sites lacking these characteristics, untangling true climate signals from tree competition and local disturbance within annual growth rings is difficult.

Detecting dendroclimatic trends in the Northeast region of the U.S., such as the Harvard Forest, is commonly problematic. An early review of dendroclimatic studies in the Norteastern U.S. by Phipps (1982) outlined many limitations that prevent the production of climate-sensitive ring chronologies due to the predominance of humid, highly competitive, young stands (historic high disturbance rates from logging and agriculture). More recent work by Pederson et al. (2012) has shown that a reasonable amount of climate information can be viably obtained from second-growth humid forests, given sufficiently robust standardization, detailed statistical analyses, and a relatively high sample number together with the use of established master chronologies. This work

showed that even humid forests are sensitive to drought stress, but that for most sites assessed a long term climate signal was not recoverable (trend > 10 years), indicating that these chronologies were not suitable for classic dendroclimatic studies (Pederson et al., 2012).

Stable isotope analysis: Physiological response of *Q. velutina* to fertilization and fertilization-climate interactions

When dendroclimatic studies fail to be sensitive enough to correlate to past climate, as is the case for many close canopied temperate forests, stable isotopes may serve as better proxies (McCarroll and Loader, 2004; Loader et al., 2008). Whereas ring width is a proxy for inter-annual variability in net tree growth, isotopes provide more specific information about how different external (e.g., temperature, soil moisture, humidity, irradiance) and internal (e.g., stomatal function, nutrient content, etc.) factors influence tree physiological responses, for which there is reasonable theory developed (Loader et al., 2008; Roden and Farquhar, 2012). Therefore, the combination of isotopes and dendroclimatology may be a more appropriate approach to assess growth and physiology of trees from humid temperate forests.

In the present study, the ability to detect significant patterns in stable isotope and iWUE response to N-fertilization was confounded by unreplicated plots, pretreatment differences, and high response variability between individual trees from the same treatment. The response ratios normalized for pretreatment differences were used in an attempt to account for some of these factors. Additionally, the approach used for selecting a sub-sample of five trees (i.e., based on highest confidence of dating accuracy)

for isotope analysis created a bias, as the mean BAI of the subsample was different from the mean BAI of the full sample of 10 trees. Consequently, the stable isotope and iWUE data may not be sufficiently representative of the entire stand to capture differences between the treatments. Another potential confounding factor was the steep increase in BAI in the control trees during the last few years of the study. The control mean BAI in T3 was more than twice that (6.1 cm^2) of the pretreatment mean BAI (2.9 cm^2) and 78% greater (3.4 cm^2) than T2 mean BAI (Fig. 1 and Fig. 1d). This growth increase in the control trees may have masked any significant treatment effect on BAI during T3 in the fertilized plots when response ratios were calculated, because the ratio directly compared the response in the fertilized plots to the control. While the increment growth increase in the control trees seems considerable, an increasing trend in *Q. rubra* growth from other parts of Harvard Forest has been observed in recent years (1993-2005; Urbanski et al., 2007).

Despite the confounding factors mentioned above, physiological responses from N fertilization were noticeable in the studied *Q. velutina* trees at Harvard Forest. iWUE was significantly enhanced in the low-N plot with a clear stimulation in photosynthesis, and although not significant in the high-N plot the same trend was observed. This effect was evident when the Scheidegger et al. (2000) conceptual model was applied to the stable isotope data of the treatment plots normalized against the control data (Fig. 4), and is supported with evidence that treatment trees had significant increases in biomass (BAI) and photosynthetic capacity reflected in the elevated foliar N content. N fertilization frequently improves iWUE by stimulating A, as plant N supply and photosynthesis are tightly coupled (Seigwolf et al., 2001; Ripullone et al., 2004; Brooks and Coulombe,

2009; Guerrieri et al., 2009; Guerrieri et al., 2010; Walia et al., 2010; Brooks and Mitchell, 2011; Guerrieri et al., 2011). The majority of these studies, however, documented transient improvements in iWUE, as most experiments were short-term (one to several field seasons) or involved pulse applications of fertilizer. The present results from the Chronic N plots are therefore unique not only because these trees received continual fertilization for 23 years, but also because there is evidence of a long-term trend of increasing iWUE over the control. In a study of similar scope, Beston et al. (2007) analyzed foliar $\delta^{13}C$ from *Pinus sylvestris* trees that had received annual N fertilization at three different application rates over a 32-year period. These trees experienced an initial minor improvement in iWUE independent of fertilization rate that was then maintained throughout the study period, but was attributed to being primarily due to changes in g_s rather than A because $\delta^{13}C$ was strongly correlated with hydroclimatic parameters. Contrastingly, in our Chronic N plots iWUE continued to improve in the fertilized plots into the most recent years of the study, although only 23 years of post-treatment data are available to date. Several factors could have contributed to this sustained increase at Harvard Forest, including selective sampling of healthy trees (already discussed), the nutrient status of the plots prior to fertilization, and negative effects as the system approaches N saturation. Magill et al. (1997) suggested that the hardwood stand where the chronic N plots are located demonstrated relatively strong N limitation prior to fertilization as it had below average N mineralization rates compared to other deciduous New England forests, potentially causing those trees to have a greater capacity to utilize additional N relative to more fertile sites. Additionally, over the study period, accelerated mortality was observed in the high-N plot, which likely released canopy space that may

have increased light interception by healthy trees. Brooks and Mitchell (2011) attributed a larger increase in $\delta^{13}C$ to a stimulation in *A* from higher light intensities reaching a greater proportion of the canopy in trees that had been fertilized and thinned compared to trees that had only received fertilizer. Fifteen years into the experiment at the Chronic N plots, tree mortality in the high-N plot was 48.9% compared to 26.6% and 18.9% in the control and low-N plot (respectively; Magill et al., 2004), suggesting that especially in the high-N plot, mortality could have contributed to a larger stimulation in *A* for the remaining healthy trees by releasing canopy space. Consequently, the combination of improved light availability and foliar nutrient status in the healthy *Q. velutina* trees in the high-N (and to lesser extend low-N) plot may, in part, explain the sustained enhancement in iWUE.

In addition to its affect on mortality and competitive dynamics, N fertilization can also lead to changes in C allocation and structural relationships in the trees, namely a reduction in the root:shoot ratio. Root biomass was documented to have decreased by 25% in both the low- and high-N plots with synchronous increases in litter production (Magill et al., 2004), indicating that C allocation in fertilized trees shifted towards a greater emphasis on aboveground biomass production. This phenomenon increased foliar N content and a shift in C allocation to favor aboveground biomass production following N fertilization has been widely documented (Brooks and Coulombe, 2009; Seigwolf et al., 2001; Balster et al., 2009; Ewers et al., 2000).

However, adjustments in whole-plant C partitioning can come at the risk of increasing vulnerability to drought events when aboveground investments are not supported by belowground hydraulic functions. This has been observed in other studies

where fertilized trees can show higher correlations compared to unfertilized trees between stable isotopes obtained from annual tree rings and corresponding climatic parameters, such as seasonal rainfall, soil moisture deficient, or relative humidity (Betson et al., 2007). Greater interannual fluctuations in isotope data during hot and dry years were interpreted by Brooks and Coulombe (2009) as indicating reduced resilience of fertilized trees to drought, which resulted in a lower capacity to maintain physiological balance during moisture-stressed conditions. Similarly, in the present study, *Q. velutina* trees in the high-N plot, and to a lesser extent in the low-N plot, showed evidence of increasing sensitivity to hydroclimatic parameters indicative of drought stress. The high-N trees had the strongest relationship between summer climatic parameters and $\delta^{18}O$ (Table 3), while the control and low-N plot showed a moderate or no relationship to $\delta^{18}O$, respectively. This could be indicative of a diphasic response, where stomatal conductance is sensitive to both low and high N availability. Moderate N fertilization could buffer against the influence of climate on stomatal conductance (expressed as reduced sensitivity to climate parameters compared to the control plot), but high levels of N-fertilization could cause a hydraulic imbalance from shifts in C allocation and increase the sensitivity of stomatal conductance to climate conditions, particularly fluctuation in air humidity or soil moisture. Another indication of the relationship between N fertilization and increasing climate sensitivity is the reduction in $\delta^{18}O$ during T3 relative to T1 and T2. Although this trend was observed across trees in all of the study plots, it was most pronounced in the fertilized trees. The change in $\delta^{18}O$ between T2 and T3 was -2.63%, -4.55% and -3.83% in the control, low- and high-N plots respectively. $\delta^{18}O$ is primarily influenced by variations in RH, but comparing the magnitude of change

between the fertilized trees and the control could indicate a response that is above that expected from climate alone, thus providing additional support for the possibility increasing drought sensitivity in response to N fertilization.

Moreover, a paradoxical positive relationship between September precipitation and $\delta^{18}O$ was found across all treatment plots (including the control). The xylem anatomy of *Quercus* spp. is optimized for high rates of water transport when water is plentiful (Abrams, 1990); therefore, typically an inverse relationship is expected whereby greater precipitation is associated with lower $\delta^{18}O$ values and greater stomatal conductance. This inverse relationship was observed in the present study, specifically between $\delta^{18}O$ and early growing season precipitation (May-July); however, this changed to a positive relationship in September whereby an increase in $\delta^{18}O$ (i.e., lower stomatal conductance) was associated with high late-season precipitation. Examination of the climate record revealed a significant trend of decreasing RH in August and September ($p < 0.01$ between T1 and T3), which may have caused stomatal closure in *Q. velutina* trees despite the trend of increasing precipitation observed during the same period. As this sensitivity in the stable isotope data to September precipitation was observed across all plots, we interpret this as being broadly indicative of a response of *Q. velutina* trees to greater moisture stress caused by declining atmospheric RH.

Typically humid temperate ecosystems, such as Harvard Forest, are not considered water-limited and therefore water availability is not usually implicated as constraining tree growth. However, recent work on tree growth and physiology has detected drought stress in many forest ecosystems including humid temperate and tropical forests (Phillips et al., 2009; Allen et al., 2010; Pederson et al., 2012). For

instance Pederson et al. (2012) detected long-term trends of interannual reductions in growth in a humid temperate forest correlated with precipitation (over the last 3-4 centuries), while severe mortality events over the past 2-3 decades have been detected worldwide, including both temperate and tropical forests, in response to acute drought events believed to be a consequence of rapid warming from climate change (Allen et al., 2010 and references therein). Additionally, a global review on tree water-transport systems found that trees are well adapted to historic variations in water availability that is typical of their region, but increased incidents of hydraulic failure are projected as species are stretched past their "safety margin" as climate change outpaces a species' ability to adapt (Choat et al., 2012). Plant safety margins against hydraulic failure were reportedly similar across sites with a wide range of mean annual precipitation, indicating that all forest ecosystems are at risk for drought-induced injury and death (Choat et al., 2012). Global trends of increased occurrence of drought stress across biomes support the possibility of late season drought conditions at Harvard Forest influencing tree physiology, and may become more pronounced if precipitation patterns change and RH continues to decline in the future.

CONCLUSION

The *Q. velutina* trees in the Chronic N Amendment plots showed a sustained improvement in growth and iWUE in comparison to the control trees over a 23-year period of N fertilization. The degree and longevity of this enhancement is exceptional in comparison to many other long-term N deposition studies from the Northeast (Elvir et al., 2003; Magill et al., 2004 (pine stand); McNulty et al., 2005; Wallace et al., 2007), especially considering that the fertilization rate in the high-N plot is unprecedentedly large (~19 times greater than ambient levels). This represents the maximum potential enhancement from chronic N deposition as this study only assessed healthy trees. While no formal evaluation took place to measure other plot characteristics, visual examination indicated that mortality was apparently much higher in the fertilized plots (especially in the high-N plot); a trend that was also documented in previous studies (Magill et al., 2004). The largest stimulation in growth was observed in the high-N plot, while the low N-plot showed the largest stimulation in iWUE. N fertilization stimulated photosynthesis (*A*), resulting in an improvement in iWUE, which was supported by Scheidegger et al. (2000) model and elevated foliar N content. There was also evidence that N fertilization altered the influence of climate on *Q. velutina* growth and iWUE. The dendroclimatic analysis revealed that fertilized trees exhibited a positive correlation between growth and late summer precipitation, while trees in the high-N plot also showed a strong correlation between $\delta^{18}O$ and summer temperature and precipitation indicating that climate strongly regulated stomatal conductance. In contrast, a positive association found in *Q. velutina* trees across all the study plots with September precipitation and $\delta^{18}O$ (more rain

decreases stomatal conductance) could be indicative that the trees are sensitive to the increasing dry air (reduced RH) at the end of the growing season. These findings add to the growing body of evidence that drought sensitivity can be detected in trees from humid temperate ecosystems.

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